



Nitrogen Processing Efficiency of an Upper Mississippi River Backwater Lake

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PURPOSE: Proximate goals of this research were to quantify the rates of nitrate removal, identify processes responsible for nitrogen transformations, and determine patterns of nitrogen loss from a backwater lake of the Upper Mississippi River (UMR). The long-range goal was to determine the management potential for removing nitrogen from the UMR by increasing flows of nitrogen-rich main channel water through backwater lakes.

BACKGROUND: Excessive nitrogen (N) fertilization and runoff from large agricultural watersheds in the UMR basin contribute substantially to eutrophication and hypoxia of freshwater and coastal marine systems (Nixon 1995). In-stream microbial processes that promote removal and dissipation of nutrients and organic carbon offer a management-based means of reducing nutrient delivery to sensitive freshwater and marine ecosystems. For instance, nitrate can be removed biologically from aquatic and terrestrial systems through microbially mediated transformations of ammonium to nitrate (nitrification) and nitrate to inert nitrogen gas (denitrification) or direct denitrification of nitrate from the water column. Recent research has shown that backwater lakes and wetlands of the UMR (Richardson et al. 2004) and other large floodplain rivers (e.g., Platte, Garonne, Seine, Swale-Ouse) have tremendous potential to remove nitrate via denitrification (Sjodin et al. 1997, Garcia-Ruiz et al. 1998, Pattinson et al. 1998, Bendjoudi et al. 2002, Baker and Vervier 2004), but the realization of this potential is often limited by the lack of delivery of N-rich water to these habitats. Because there are operational opportunities to direct or increase flows to many backwater areas of the UMR it is pertinent to determine the true potential to affect improved N retention through increased connection between the main channel and backwater lakes. More information is needed regarding N processing efficiency of backwater systems in order to determine if increased loading of N-rich water to backwaters will be an effective management strategy for reducing N transport to coastal systems. The question that needs to be addressed is whether large river systems and waterways can be managed for both reductions in watershed N loading and encouragement of in-stream N dissipation via nitrification-denitrification processes.

The objectives of this study were to examine N processing, fate, and removal efficiency in a backwater system of the UMR that receives N loads from the main channel via a controlled culvert structure.

STUDY SITE: Third Lake is a part of the interconnected Finger Lakes backwater system (Clear, Lower Peterson, Schmokers, Third, Second, and First Lakes) located in navigation pool 5, immediately downstream of the Lock and Dam 4 dike on the Upper Mississippi River

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(Figure 1). Third Lake is very shallow (mean depth = 0.6 m and maximum depth = 2.0 m at the nominal pool elevation of 201.2 m MSL) and small, with a surface area of 11 ha and a volume of 67,500 m³. It is currently eutrophic (chlorophyll = 55 mg m⁻³; total P = 0.082 mg L⁻¹; soluble reactive P = 0.041 mg L⁻¹) and nitrogen species entering the lake are dominated by nitrate-nitrite-N (total N = 3.53 mg L⁻¹; NO₃NO₂-N = 2.56 mg L⁻¹). Dense stands of submersed and emergent aquatic plants cover ~ 60 percent of the lake's surface area, dominated by *Ceratophyllum demersum*, *Myriophyllum spicatum*, and *Nymphaea odorata*.

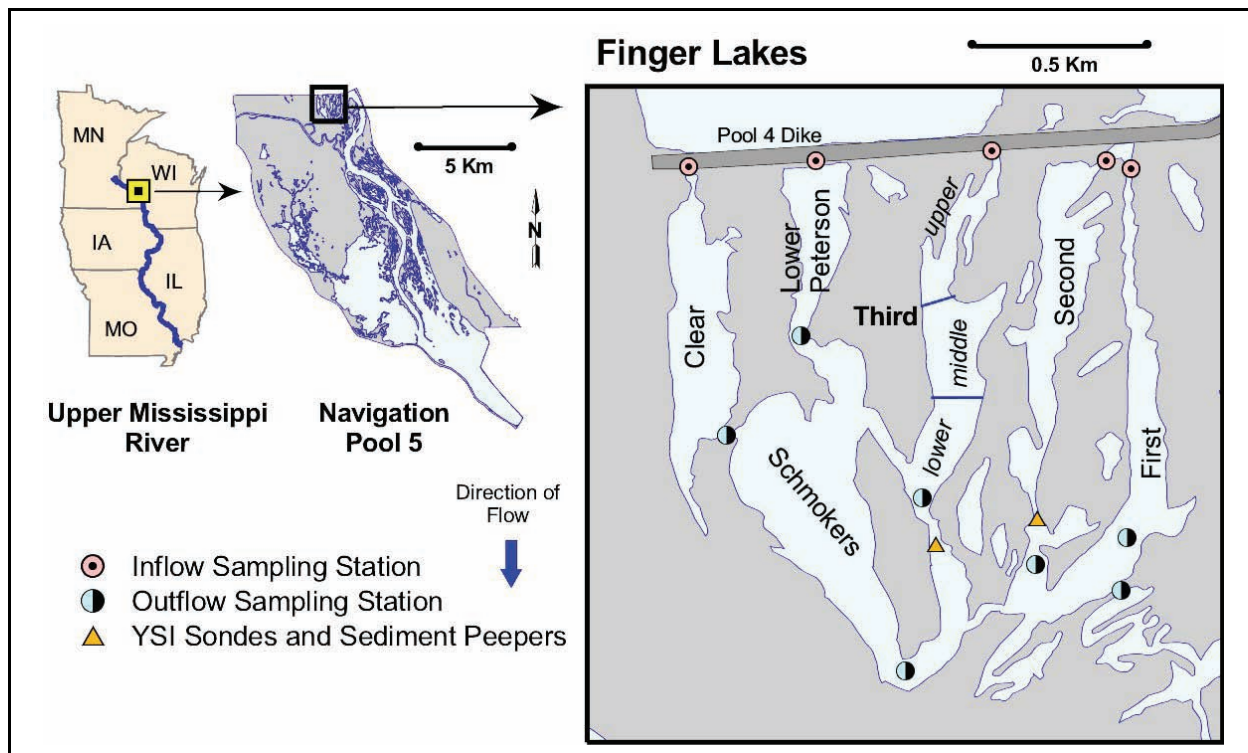


Figure 1. Location of the Finger Lakes in pool 5 of the Upper Mississippi River

Prior to 1965, direct flow from the Mississippi mainstem into the Finger Lakes was prevented by the Lock and Dam 4 dike. Water exchanges occurred primarily during floods on the Mississippi and Zumbro Rivers. The latter tributary drains to the south of the backwater complex, and bank overflows during storm discharges provided water exchanges with the backwater system. In 1965, a 1.2-m-diam culvert was installed through the dike to allow discharge from navigation Pool 4 into Lower Peterson Lake (Johnson et al. 1998). Concerns over lack of water exchanges and poor dissolved oxygen conditions in winter led to the installation of additional culverts in 1994 to supply flows into the other lakes downstream of the dike. Each culvert system was fitted with adjustable vertical slide gates to control flows within a range of 0 to 2.8 m³ s⁻¹, depending on culvert size. Third Lake currently receives flow through a 0.9-m-diam circular culvert and flows are typically maintained at ~0.1 m³ s⁻¹ in winter (November through April) and ~1 m³ s⁻¹ in summer (maximum culvert flow = ~1.6 m³ s⁻¹).

METHODS: Sampling stations, established at the culvert inflow and outflow of Third Lake, were visited at weekly intervals between May and September 2004 (Figure 1). Water depth at

the outflow station was ~ 0.4 m at nominal pool elevation. Additional sampling stations, established along longitudinal and transverse axes of the lake, were sampled at biweekly to monthly intervals (Figure 2). Culvert flows were measured using a Flo-Mate model 2000 velocity meter (Marsh-McBirney Inc., Fredrick, MD). In situ characteristics (i.e., water temperature, dissolved oxygen, pH, and conductivity) were measuring using a Hydrolab Quanta monitoring system (Hach Co., Loveland, CO) that was precalibrated against known standards and independently determined Winkler dissolved oxygen. Surface water grab samples collected at each station were analyzed for total and total soluble nitrogen (N), nitrate-nitrite N ($\text{NO}_3\text{NO}_2\text{-N}$), and ammonium N ($\text{NH}_4\text{-N}$). Soluble N constituents were filtered through a 0.45- μm membrane filter in the field and preserved on ice until analysis. Samples for total and total soluble N determination were digested with potassium persulfate according to Ameel et al. (1993) prior to analysis. Chemical analyses were performed on a Lachat QuikChem A/E using standard procedures (American Public Health Association (APHA) 1998).

Daily navigation pool 4 and 5 stage elevations and tailwater flows and elevations for Lock and Dam 4 were obtained from the U.S. Army Engineer District, St. Paul (St. Paul, Minnesota). Stage elevation was monitored on Third Lake at 15-min intervals between late June and September 2004 using a data logging system equipped with a pressure transducer (ISCO Model 4120; Teledyne ISCO, Inc., Lincoln, NE). $\text{NO}_3\text{NO}_2\text{-N}$ discharges from navigation pool 4 were estimated using concentration information collected as part of another study from Upper Peterson Lake near its discharge into First Lake.

For inflow-outflow budget analysis, culvert loading of total and soluble constituents was calculated as the product of flow and concentration. Outflow constituent discharge was estimated as the product of culvert flow (i.e., culvert inflow ~ water discharge from the lake) and concentration was measured at the outflow station. Groundwater influx (e.g., dike seepage) into the system was not measured and these fluxes were not included in the mass balance

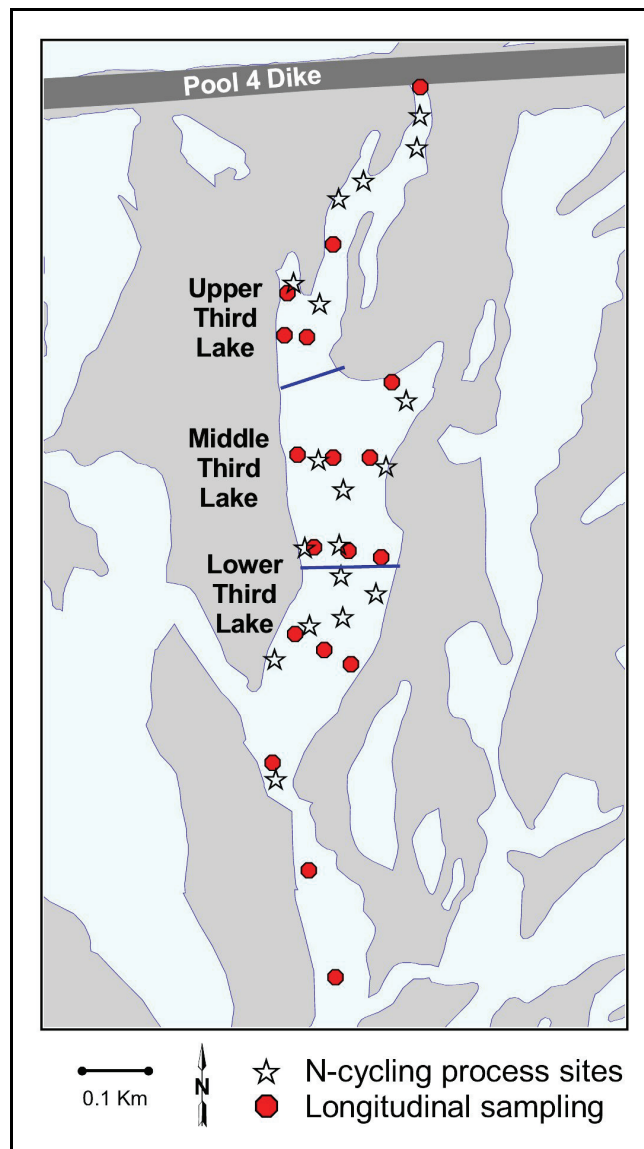


Figure 2. Sampling station locations for examination of longitudinal nitrogen gradients in the water column and sediment nitrogen dynamics

calculations. Net retention (positive) or export (negative) of constituent loads was calculated as inflow load minus outflow discharge.

Components of the nitrogen cycle, including nitrification and denitrification, as well as sediment and macrophyte total N and C, and particulate organic carbon (POC), were determined for inclusion into the N budget and for quantification of causal mechanisms of nitrate and ammonium loss and gain. Process measurements were conducted on 28 May and 22 July, to bracket the early and maximal growth period for aquatic plants, cool- and warm-water effects on microbial N cycling, and seasonally high and low $\text{NO}_3\text{NO}_2\text{-N}$ loading. N-cycling in sediments was focused on because previous work showed C-rich sediments of UMR backwaters to be biogeochemical hotspots for N-cycling (Richardson et al. 2004). Significant N-cycling can also occur in the epiphytic microbial community on macrophyte stems and leaves and by the macrophytes themselves and the authors intend to measure these levels in subsequent studies.

Six randomly selected stations were established in the upper, middle, and lower third of the lake for process sampling purposes (Figure 2). Water temperature, specific conductivity, dissolved oxygen, and pH were measured in situ at each station with a YSI 600XL multiparameter probe. Measurements were made mid-depth for sites <1.5 m deep and at 30 cm above the sediments and 30 cm below the water surface for sites >1.5 m. Submersed aquatic macrophyte biomass was quantified at each station in May and July by harvest of all plant material within a 0.5-m^2 sampling frame (one sample per station). Macrophytes were sorted and identified, oven dried (105°C for 24 hr, Wetzel and Likens (1991)) to constant mass. Sub-samples of dried plant tissue were ground to a coarse powder using a Wiley mill with a $420\text{-}\mu\text{m}$ screen, then combusted in a Variomax Elemental Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ) to determine total C and N content.

Six to eight sediment cores (upper 5 cm x 7.62 cm diam) were collected at the same stations in May and July (Figure 2). Sediment pH and temperature were measured immediately after collection with a Beckman Φ pH meter and then refrigerated for later processing. Sediment total C and N were determined from subsamples using a Variomax Elemental Analyzer. Sediment porewater was removed from another subsample by centrifugation at 3000 rpm for 12 min for analysis of $\text{NH}_4\text{-N}$ using the phenol hypochlorite method (Solorzano 1969) and automated analytical techniques (Bran+Luebbe TrAAcs 800 Continuous Flow Analysis System according to standard methods; APHA (1998)). From another subsample, sediment was extracted for 1 hr with a 1 N potassium chloride solution following Caffrey and Kemp (1992) for determination of sediment exchangeable $\text{NH}_4\text{-N}$. The extractant was separated from the sediment slurry by centrifugation, filtered through a $0.45\text{-}\mu\text{m}$ filter, and analyzed for $\text{NH}_4\text{-N}$ using the automated techniques described above.

Denitrification was estimated in sediment slurries from the top 5 cm of each of four cores using the acetylene block method (Sorensen 1978, Tiedje et al. 1989). Ambient denitrification rates (Table 1) were estimated with slurries (24 to 40 g wet mass) constituted with only site water (= U-DEN); carbon limitation (+C) was estimated with addition of soluble C (final concentrations: $12\text{ mg glucose-C} \cdot \text{L}^{-1}$); nitrate limitation (+N) was estimated with addition of nitrate (final concentration $14\text{ mg NO}_3^- \cdot \text{N} \cdot \text{L}^{-1}$); and addition of both C and N estimated the total enzyme potential for denitrification under optimal conditions (denitrification enzyme activity; = DEA; Groffman et al.(1999)). A fifth metric of denitrification, estimated denitrification rate

(= EDR), was calculated using DEA, U-DEN, and nitrification rates (see method below). EDR is considered a more realistic estimate of actual denitrification because it accounts for the nitrate that is produced through nitrification (Richardson et al. 2004).

Table 1 Description of Denitrification Rate Metrics		
Denitrification Metric	Abbreviation	Process Description
Ambient denitrification rate	U-DEN	Rate determined under in situ temperature conditions and nitrate + organic carbon availability
Denitrification enzyme activity	DEA	Rate determined at a standard temperature with no organic carbon or nitrate limitation (i.e., assays spiked with sufficient N and organic C to prevent enzyme activity limitation)
Estimated denitrification rate	EDR	Coupled nitrification and denitrification accounted for in the calculation. Nitrate produced via nitrification is included as a source for denitrification, in addition to water column concentrations

After slurries were prepared for denitrification rate determination, anaerobic conditions were created through purging of sample jars of oxygen with scrubbed, ultra-high purity helium for 15 min. Atomic absorption grade acetylene (20 mL) was then added with a syringe through a septum on the top of each sample container. Slurries were incubated under constant agitation (175 revolutions per minute) at ambient river temperatures in a darkened incubator. Headspace gas was sampled at 30, 60, and 90 min and nitrous oxide (N₂O) concentrations were measured using a Hewlett-Packard Model 5890 gas chromatograph with an electron capture detector (ECD ⁶³Ni).

Gross nitrification rate measurements were initiated on sediment slurries (upper 5 cm of sediment from cores collected at each station) within 24 hr of sample collection using a modification of the nitrapyrin method described by Strauss and Lamberti (2000) and Strauss et al. (2004). For each station, sediment slurries (25 to 40 g wet mass) were incubated in two 125-mL Erlenmeyer flasks with 81 mL of unfiltered river water collected from each station. Nitrification was inhibited in one sediment incubation flask by addition of nitrapyrin (2-chloro-6-[trichloromethyl]-pyridine, SIGMA Chemical Co., St. Louis, Missouri) dissolved in dimethyl sulfoxide (DMSO). The final concentration of nitrapyrin was 10 mg L⁻¹. Nitrification was allowed to occur in the other flask and it received DMSO only to serve as a control. The flasks were covered loosely with aluminum foil and incubated in the dark at ambient river temperature for 72 hr on an orbital shaker (175 rpm). Initial and final sediment exchangeable NH₄-N concentrations were determined from subsamples collected from each flask by extraction. Gross nitrification rates over the incubation period were calculated by subtracting the observed change in sediment exchangeable NH₄-N in the flasks containing only DMSO from the increase in NH₄⁺ in the flasks that contained nitrapyrin plus DMSO. This method may overestimate nitrification to some extent because the laboratory incubations were conducted under ideal conditions (i.e., aerobic and well-mixed).

Differences in mean nutrient concentrations and N-cycling rates, between sampling dates and among regions of Third Lake, were tested using two-way Analysis of Variance (ANOVA) with

SAS® software, General Linear Models (Littell et al. 1991). In the ANOVA, a significant interaction effect indicates statistical dependency of location effects and sampling date. Probability values reported for tests of statistical significance in this report refer to ANOVA results unless otherwise noted.

RESULTS

Nitrogen Removal Capacity and Efficiency.

Between late May and mid-June, Lock and Dam 4 tailwater elevations increased due to storm-related flows from the Mississippi and Zumbro Rivers (Figure 3). Water surface elevation at Third Lake was similar to the Lock and Dam 4 tailwater during this period, indicating flooding and water exchanges between the Finger Lakes and the Mississippi and Zumbro Rivers. During other summer periods, Third Lake pool elevation was nearly constant and slightly higher than that of the Lock and Dam 4 tailwater.

$\text{NO}_3\text{NO}_2\text{-N}$ concentration and loading for the Mississippi River at Lock and Dam 4 exhibited a distinct seasonal pattern (Figure 4). In early May, the concentration was less than $0.5 \text{ mg} \cdot \text{L}^{-1}$. It increased substantially in late June and, to a lesser extent, in late July in conjunction with storm-related peaks in Mississippi River flow. Concentration maxima typically lagged behind peak flows by ~ 7 days during these periods. As flows subsided in late July through August, $\text{NO}_3\text{NO}_2\text{-N}$ declined in concentration to a minimum in early September. Concentration increases occurred in late September as a result of storm-related flow increases on the Mississippi River.

During periods of nominal tailwater elevation at Lock and Dam 4, water inflow to Third Lake was primarily via culvert discharge and flows were nearly constant at $\sim 0.6 \text{ m}^3 \cdot \text{s}^{-1}$ ($\pm 0.05 \text{ S.E.}$; Figure 4). The water residence time (lake volume \div flow) for Third Lake during these periods was ~ 2 days ($\pm 0.2 \text{ S.E.}$). Increases in the Lock and Dam 4 tailwater elevation during high

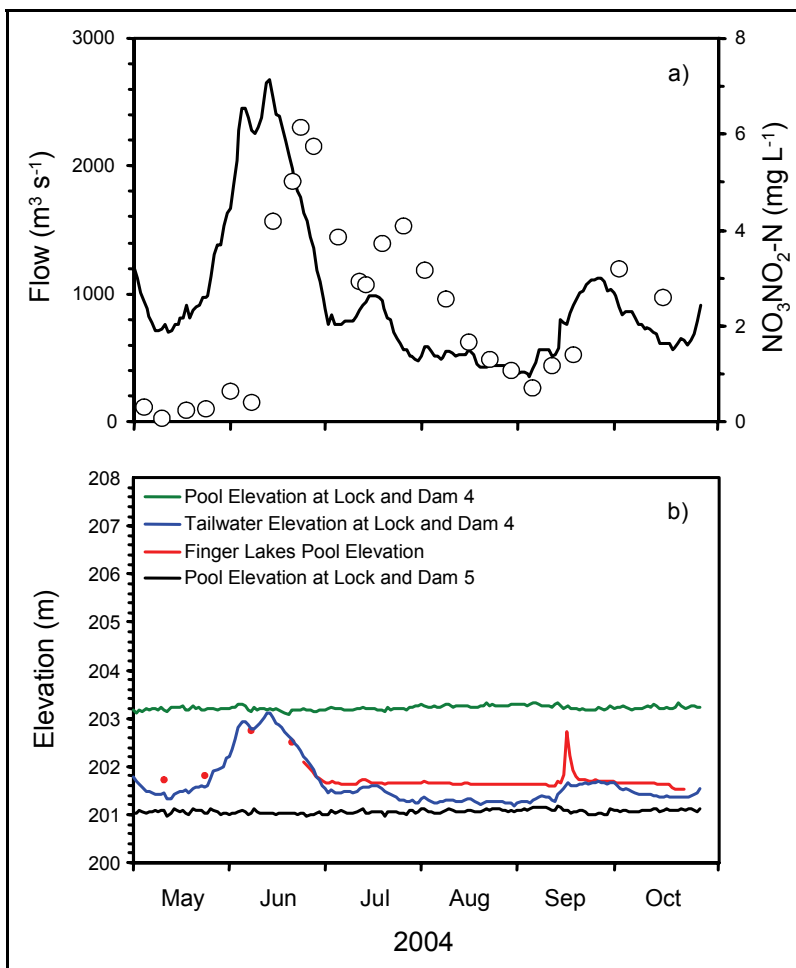


Figure 3. Seasonal variations in (a) tailwater flow below Lock and Dam 4 and nitrate-nitrite nitrogen ($\text{NO}_3\text{NO}_2\text{-N}$) concentration and (b) pool elevation

Mississippi and Zumbro River flows dominated water inflow to Third Lake in late May through June. Culvert flow and water residence time for the lake could not be accurately determined during that period.

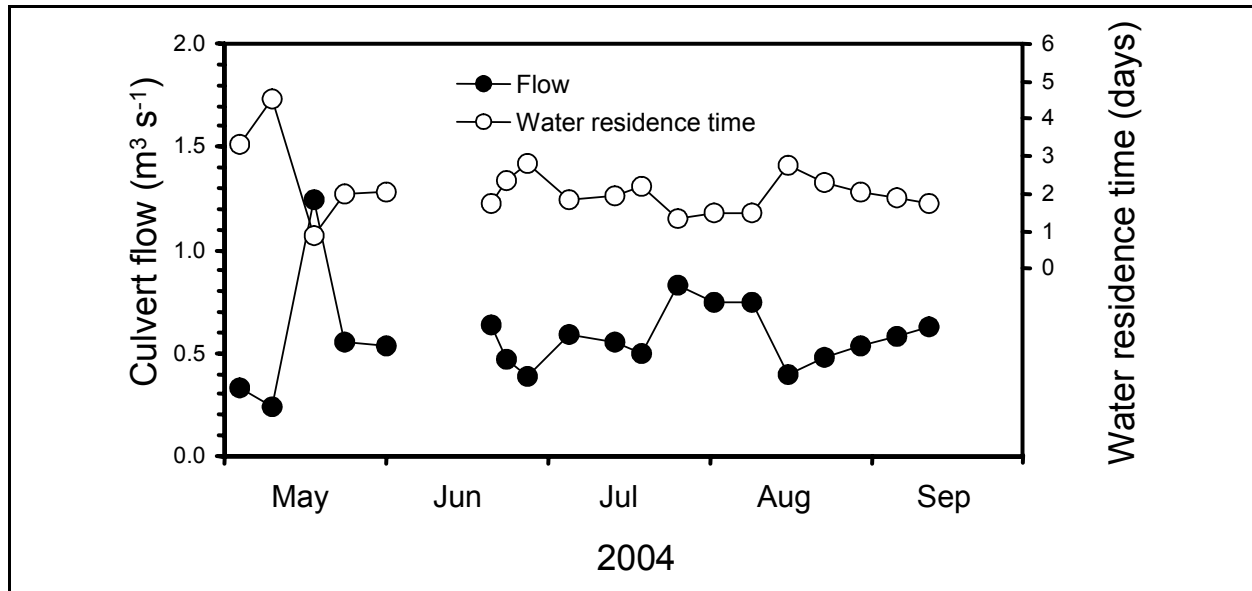


Figure 4. Seasonal variations in culvert flow and water residence time for Third Lake

In May, culvert inflow concentrations of total N were low relative to other summer periods (Figure 5). They increased substantially during high Mississippi and Zumbro River flow between late May and June. However, the outflow station of Third Lake had total N concentrations that were higher than the culvert inflow during this period because of water and constituent influxes into Third Lake from the Zumbro River, which backed into the lake during the flood. The very high total N concentrations in the Zumbro River during the flood ($> 12 \text{ mg} \cdot \text{L}^{-1}$) account for the high total N concentrations observed at the outflow station. Total N inflow concentrations at the culvert declined between late June and mid July, increased in late July in conjunction with a smaller peak in Mississippi River discharge, and steadily declined between August and early September.

Total N loading through the culvert to Third Lake varied primarily as a function of total N concentration of the inflow in early May and between late June and September, because culvert flows were nearly constant during these periods (Figure 5). Total N concentrations and discharges at the outflow station were lower than total N culvert loads, indicating net retention of total N in the lake over most of the summer. Greater net total N retention ($\text{kg} \cdot \text{d}^{-1}$) occurred as a linear function of greater total N culvert loading ($\text{kg} \cdot \text{d}^{-1}$) to the lake (net total N retention = $\{0.3172 \cdot \text{total N load}\} - 2.443$; $r^2 = 0.84$). Net total N retention efficiency (i.e., $\{\text{net total N retention} / \text{total N culvert load}\} \cdot 100$) was 31 percent for the summer period (Table 2).

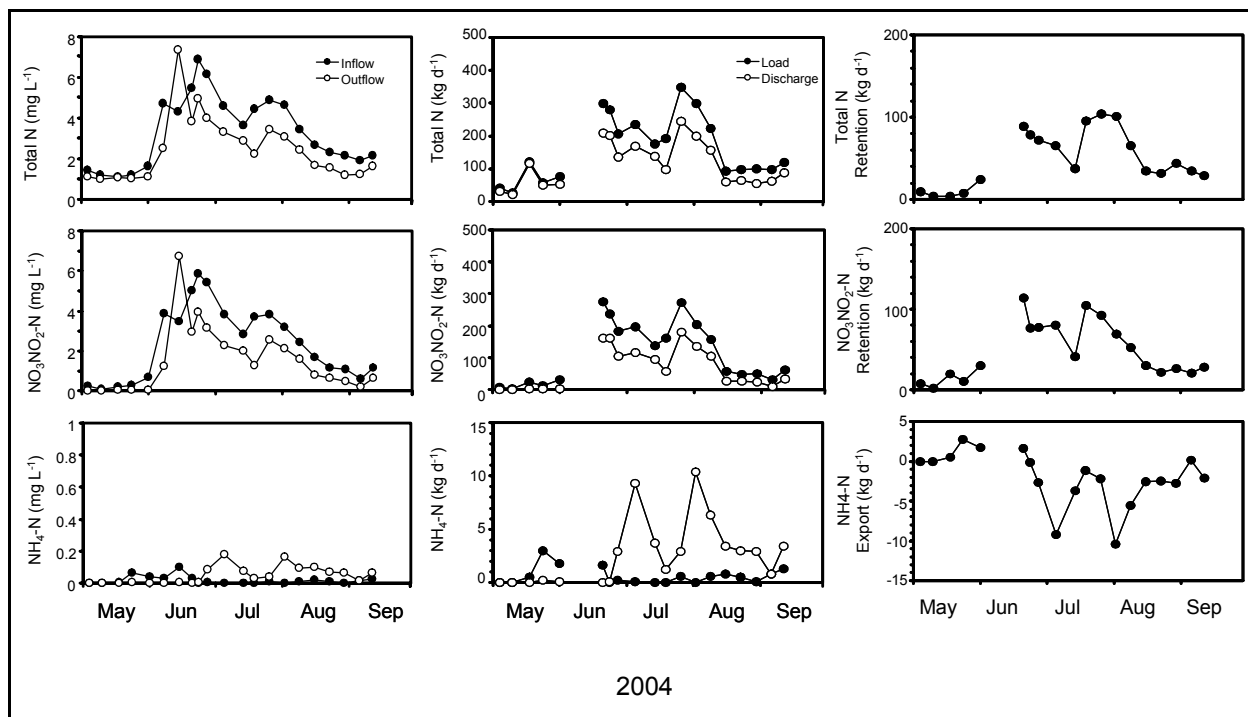


Figure 5. Seasonal variations in (upper row) total nitrogen (N), (middle row) nitrate-nitrite N ($\text{NO}_3\text{NO}_2\text{-N}$) and ammonium N ($\text{NH}_4\text{-N}$) concentration, load/discharge, and retention (positive flux) or export (negative flux) from Third Lake

Variable	Culvert Loading (kg d^{-1})	Discharge (kg d^{-1})	Net Retention/Export (kg d^{-1})	Net Retention/Export (%)
Total N	163	113	50	30.7
$\text{NO}_3\text{NO}_2\text{-N}$	113	65	48	42.5
$\text{NH}_4\text{-N}$	0.6	2.7	-2.1	-350.0
PON	24.6	17.7	6.9	28
DON	24.3	26.6	-3.3	-13.6

¹ $\text{NO}_3\text{NO}_2\text{-N}$ = nitrate-nitrite-N. $\text{NH}_4\text{-N}$ = ammonium-N, PON = particulate organic N, DON = dissolved organic nitrogen.

$\text{NO}_3\text{NO}_2\text{-N}$ accounted for 7 to 92 percent of the total N culvert loading to Third Lake and, thus, exhibited very similar seasonal inflow-outflow patterns to that of total N (Figure 5). Before the Mississippi River flood, $\text{NO}_3\text{NO}_2\text{-N}$ concentrations and culvert loading in early May were very low ($0.20 \text{ mg} \cdot \text{L}^{-1} \pm 0.04 \text{ S.E.}$ and $11.1 \text{ kg} \cdot \text{d}^{-1} \pm 4.5 \text{ S.E.}$, respectively) and represented a much smaller percentage of the total N load (16 percent $\pm 3 \text{ S.E.}$). The storm-related flows resulted in

much higher concentrations and loading of $\text{NO}_3\text{NO}_2\text{-N}$ to Third Lake in June. Between late June and September, $\text{NO}_3\text{NO}_2\text{-N}$ accounted for a mean 70 percent (± 5 S.E.) of the total N load into the lake. As with total N, the concentration and load declined from a peak in late June to a minimum in September. Substantial net retention of $\text{NO}_3\text{NO}_2\text{-N}$ loads occurred throughout the summer; it also varied linearly as a function of $\text{NO}_3\text{NO}_2\text{-N}$ loading (Figure 6).

Net retention efficiency of $\text{NO}_3\text{NO}_2\text{-N}$ culvert loads to Third Lake was ~43 percent (Table 2). A log-linear relationship also existed between $\text{NO}_3\text{NO}_2\text{-N}$ culvert loading and net retention efficiency (Figure 6). It approached 100 percent at low $\text{NO}_3\text{NO}_2\text{-N}$ culvert loading. As $\text{NO}_3\text{NO}_2\text{-N}$ culvert loading increased, net retention efficiency declined and approached ~40 percent as $\text{NO}_3\text{NO}_2\text{-N}$ culvert loading exceeded $100 \text{ kg} \cdot \text{d}^{-1}$.

Longitudinal variations in $\text{NO}_3\text{NO}_2\text{-N}$ concentration in Third Lake could be described by a negative exponential decay function ($N_{(d)} = Ne^{-(k \cdot d)}$; where N = concentration, d = distance, and k = decay coefficient), as concentrations were greatest at the culvert inflow and declined with increasing distance to the outflow (Figure 7). Variations in the decay coefficient (k) were associated primarily with variations in the inflow $\text{NO}_3\text{NO}_2\text{-N}$ concentration and load (Figure 8), since culvert flows and water residence times were nearly constant on these dates. k decreased exponentially as a function of increasing $\text{NO}_3\text{NO}_2\text{-N}$ culvert load. The $\text{NO}_3\text{NO}_2\text{-N}$ uptake length ($S = 1/k$; the distance traveled before becoming incorporated; Newbold et al.(1981)) also varied as a function of $\text{NO}_3\text{NO}_2\text{-N}$ culvert load, ranging from 625 m at culvert loads of $\sim 50 \text{ kg} \cdot \text{d}^{-1}$ to greater than 1500 m (i.e., greater than the length between the culvert inflow and outflow station) as loading exceeded $200 \text{ kg} \cdot \text{d}^{-1}$.

Spatially, longitudinal gradients in $\text{NO}_3\text{NO}_2\text{-N}$ concentration developed in Third Lake in June as a result of storm-related influx of high concentrations of $\text{NO}_3\text{NO}_2\text{-N}$ from the Mississippi River (Figure 9). Lateral variations in concentration along transects located more than 400 m from

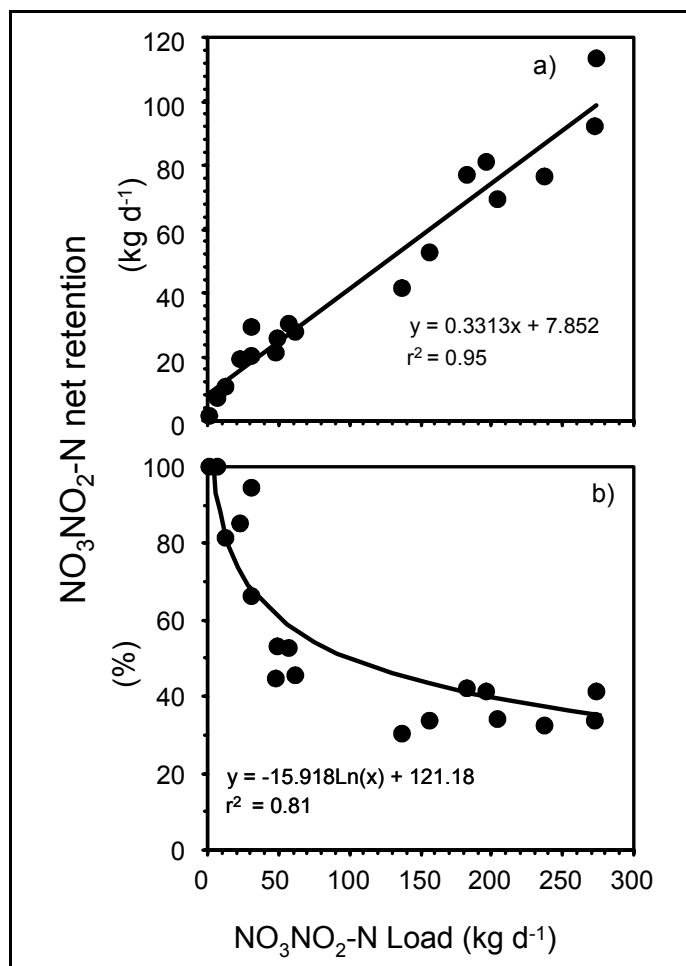


Figure 6. Relationships between nitrate-nitrite nitrogen ($\text{NO}_3\text{NO}_2\text{-N}$) culvert loading and (a) $\text{NO}_3\text{NO}_2\text{-N}$ net retention rate and (b) $\text{NO}_3\text{NO}_2\text{-N}$ net retention efficiency

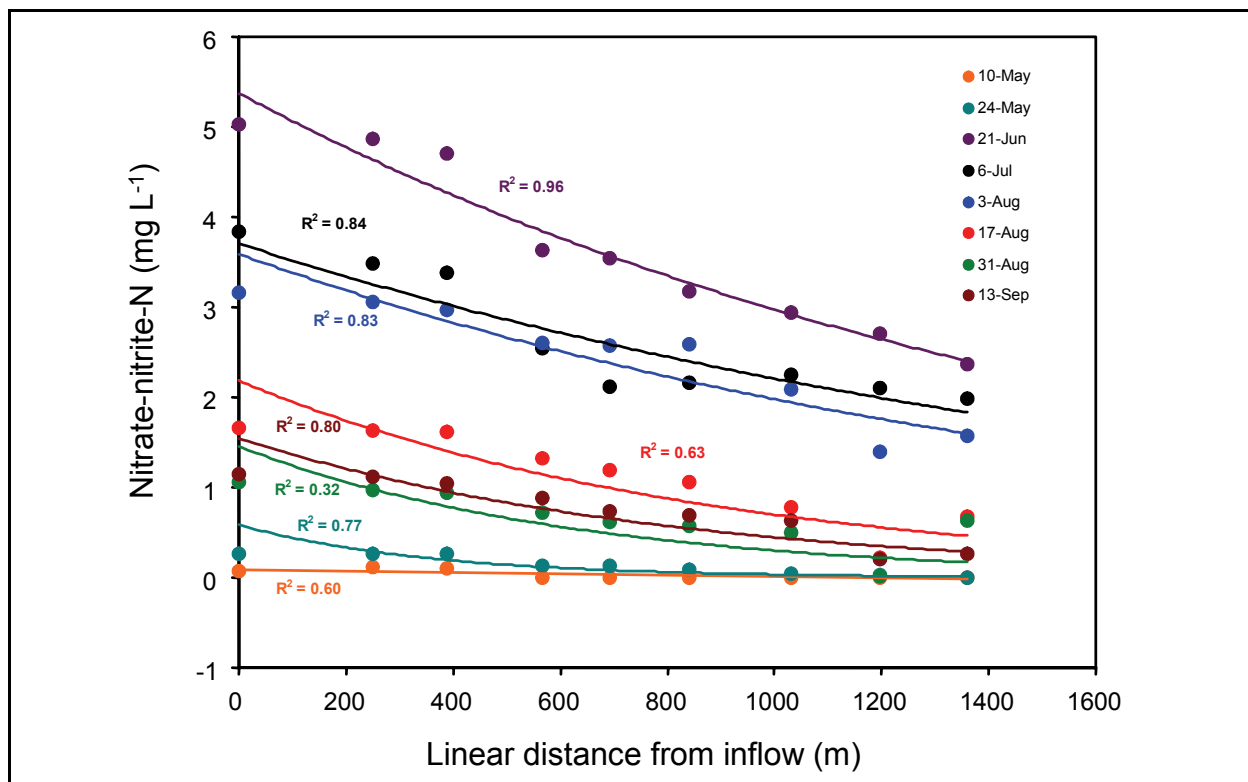


Figure 7. Variations in nitrate-nitrite nitrogen concentration as a function of distance from culvert inflow for different dates during summer 2004

the inflow were minimal on 21 June, indicating fairly complete lateral mixing as loads moved through the lake. However, concentrations were lower in the two northern embayments (i.e., located between 200 and 400 m) on this date, compared to concentrations observed in the adjacent main channel, suggesting some isolation from the bays. Between July and late August, longitudinal concentration gradients diminished in conjunction with inflow concentration declines, reflecting increases in the decay coefficient. During this same time period, accelerated $\text{NO}_3\text{NO}_2\text{-N}$ depletion occurred in the more isolated northern embayments, relative to other regions of the lake.

In contrast to total N and $\text{NO}_3\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$ was usually exported from Third Lake during the summer, particularly during July through September (Figure 5). $\text{NH}_4\text{-N}$ culvert inflow concentrations and loading were greatest during the Mississippi River flooding period between late May and June. Net retention of $\text{NH}_4\text{-N}$ culvert inflow loads occurred only during this period. During other periods (early May and late June through September), $\text{NH}_4\text{-N}$ culvert inputs were near zero and outflow concentrations and discharges from Third Lake exceeded those of the culvert inflow. Peaks in outflow $\text{NH}_4\text{-N}$ concentration and discharge were observed in early July and early August, shortly after peaks in total N and $\text{NO}_3\text{NO}_2\text{-N}$ culvert inputs. Overall, the net $\text{NH}_4\text{-N}$ export from Third Lake for the summer represented greater than 300 percent of the $\text{NH}_4\text{-N}$ culvert load (Table 2). However, this export rate was less than 2 percent of the total N composition in the outflow of the lake.

Nitrogen Fluxes, Transformations, and Fate.

Macrophyte biomass in Third Lake was dominated by *Ceratophyllum*, *Nymphaea*, and *Myriophyllum*. Other genera were present, but rarely accounted for a significant proportion of sample biomass (*Potamogeton zosteriformis*, *P. crispus*, *Myriophyllum spicatum*, *Elodea canadensis*, *Stuckenia pectinata*, *Zosterella dubia*, and *Eleocharis* sp.). Mean macrophyte biomass was significantly lower in May ($18.9 \text{ g} \cdot \text{m}^{-2} \pm 7.2 \text{ S.E.}$) than in July ($58 \text{ g} \cdot \text{m}^{-2} \pm 11.2 \text{ S.E.}$; $P = 0.0095$). However, there were no significant differences in the mean vegetation mass among the three lake regions during either period.

There was little variation in tissue N among plant taxa. Mean tissue percent N did vary significantly between seasons (May: $3.02 \% \pm 0.13 \text{ S.E.}$, July: $2.47 \% \pm 0.15 \text{ S.E.}$; $P = 0.0064$). Mean macrophyte tissue N mass doubled from $0.72 \text{ g} \cdot \text{m}^{-2} (\pm 0.27 \text{ S.E.})$ in May to $1.43 \text{ g} \cdot \text{m}^{-2} (\pm 0.28 \text{ S.E.})$ in July, and total lake tissue N in July was nearly two times that in May ($21.5 \text{ kg} \pm 4.1 \text{ S.E.}$ versus $10.7 \text{ kg} \pm 4.1 \text{ S.E.}$, respectively; $P = 0.084$). There were no significant differences in percent macrophyte tissue N among the three regions of the lake. Average lake-wide N incorporation into macrophytes during the sampling interval was $0.2 \text{ kg} \cdot \text{d}^{-1}$. C in plants averaged 33.9 percent ($\pm 1.21 \text{ S.E.}$) and was not different between sample dates, among lake regions, or plant taxa.

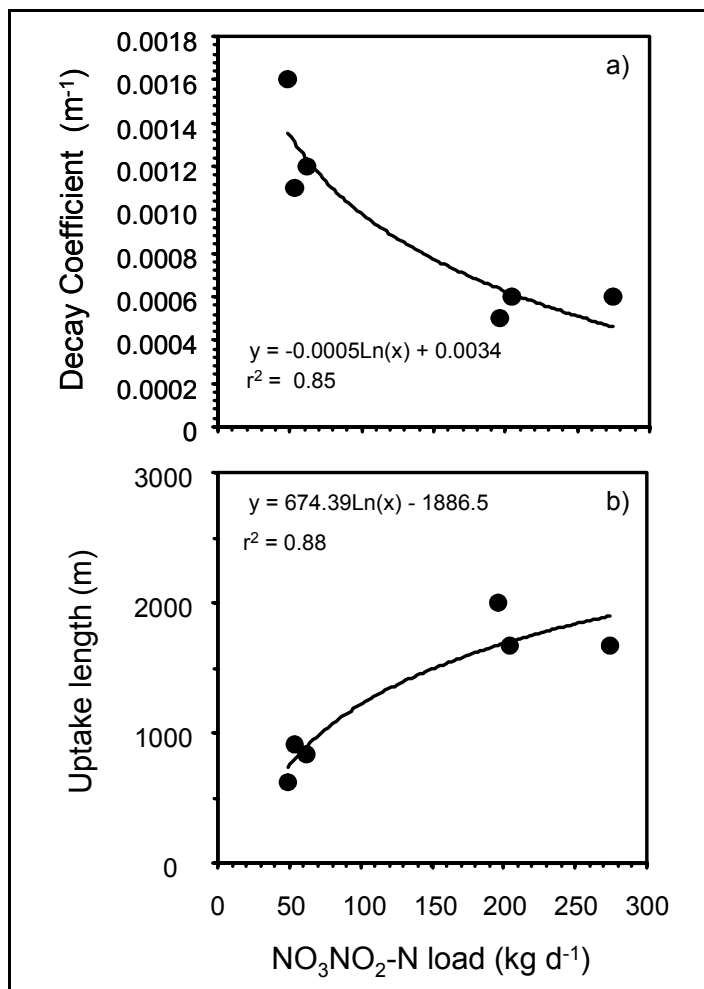


Figure 8. Relationships between nitrate-nitrite nitrogen ($\text{NO}_3\text{NO}_2\text{-N}$) culvert load to Third Lake and (a) decay coefficient and (b) uptake length

When sediment nitrification-denitrification was sampled in May, water column mean $\text{NO}_3\text{NO}_2\text{-N}$ concentrations were extremely low throughout the lake ($0.15 \text{ mg} \cdot \text{L}^{-1} \pm 0.018 \text{ S.E.}$) with no discernable spatial pattern (Figure 10). During July, however, mean $\text{NO}_3\text{NO}_2\text{-N}$ concentrations were more than 10 times higher ($1.70 \text{ mg} \cdot \text{L}^{-1} \pm 0.18 \text{ S.E.}$; $P < 0.0001$) and exhibited a distinct spatial pattern. $\text{NO}_3\text{NO}_2\text{-N}$ concentrations ($2.6 \text{ mg} \cdot \text{L}^{-1} \pm 0.09 \text{ S.E.}$) were two times higher in the upper third of the lake than in the lower two thirds ($1.3 \text{ mg} \cdot \text{L}^{-1} \pm 0.06 \text{ S.E.}$). A significant two-way interaction ($P_{\text{Event} \times \text{Region}} < 0.0001$) for $\text{NO}_3\text{NO}_2\text{-N}$ indicated the spatial variation was dependent on sampling event. Mean water column $\text{NH}_4\text{-N}$ concentrations also showed distinct spatial patterns and a significant season versus region interaction ($P_{\text{Event} \times \text{Region}} < 0.02$). Concentrations in the upper third of the lake were higher than the rest of the lake in both May

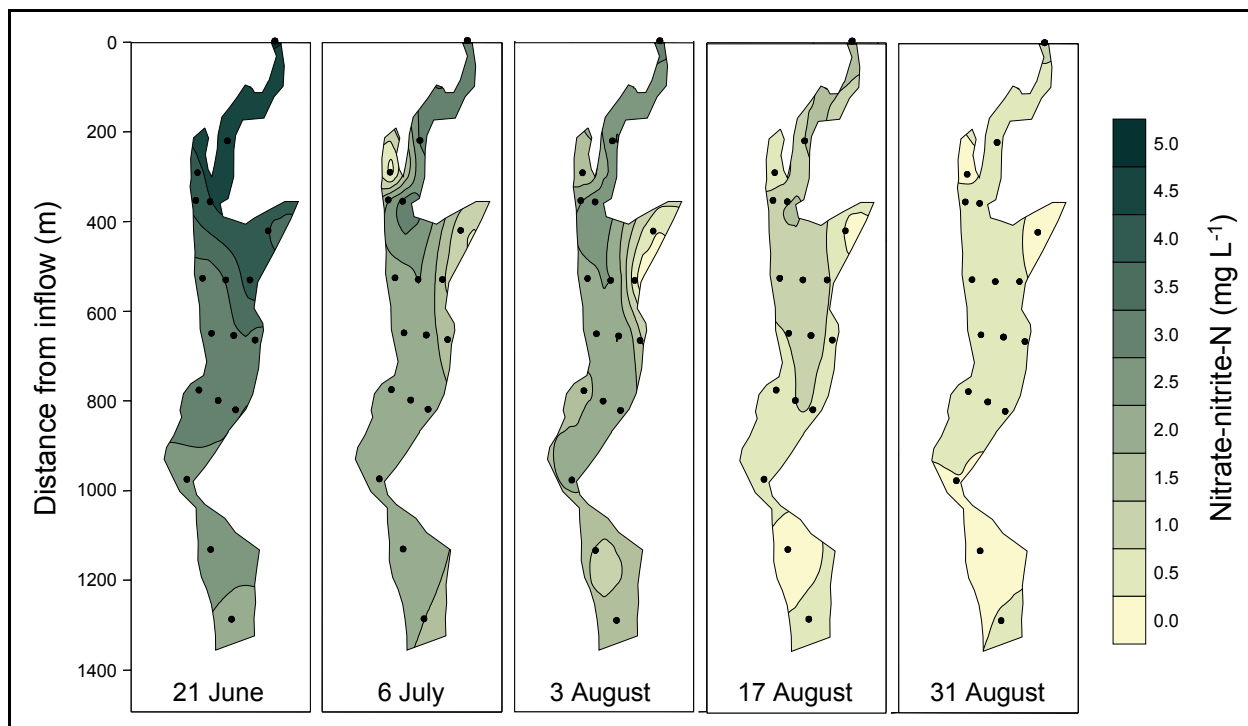


Figure 9. Spatial variations in nitrate-nitrite nitrogen concentration in Third Lake on various dates. Solid dots represent sampling locations

and July but with greatest differences in May (Figure 11). Mean $\text{NH}_4\text{-N}$ concentrations in the upper third in May were $0.12 \text{ mg} \cdot \text{L}^{-1}$ (± 0.021 S.E.), dropping to $0.03 \text{ mg} \cdot \text{L}^{-1}$ (± 0.01 S.E.) and $0.015 \text{ mg} \cdot \text{L}^{-1}$ (± 0.003 S.E.) in the middle and lower third, respectively. In July, mean $\text{NH}_4\text{-N}$ in the upper third was $0.09 \text{ mg} \cdot \text{L}^{-1}$ (± 0.021 S.E.) while mean concentration in the lower lake was $0.06 \text{ mg} \cdot \text{L}^{-1}$ (± 0.01 S.E.). Porewater $\text{NO}_3\text{NO}_2\text{-N}$ concentrations were extremely low in May ($0.002 \text{ mg} \cdot \text{L}^{-1} \pm 0.0006$ S.E.) and undetectable in July (Figure 10).

Porewater $\text{NH}_4\text{-N}$ concentrations were not different among regions of the lake or between dates, ranging from $3.9 \text{ mg} \cdot \text{L}^{-1}$ (± 2.1 S.E.) in the lower lake in May to $8.0 \text{ mg} \cdot \text{L}^{-1}$ (± 2.1 S.E.) in mid-lake in July (Figure 11). There were also no consistent patterns in exchangeable $\text{NH}_4\text{-N}$ on a volumetric basis, ranging from $18.3 \text{ mg} \cdot \text{L}^{-1}$ (± 6.8 S.E.) in the lower lake in May to $31.1 \text{ mg} \cdot \text{L}^{-1}$ (± 6.8 S.E.) in mid-lake in July. On a mass basis, however, exchangeable $\text{NH}_4\text{-N}$ was significantly higher in July ($0.13 \text{ mg} \cdot \text{g}^{-1} \pm 0.03$ S.E.) than in May ($0.07 \text{ mg} \cdot \text{g}^{-1} \pm 0.018$ S.E.; $P=0.057$). Total C content of sediments (upper 5 cm) ranged between 5.1 and 6.5 percent and the percent total sediment N ranged between 0.36 and 0.56 percent. No significant trends were observed as a function of time or space.

Mean estimated denitrification rates (EDR) (Figure 12) exhibited no seasonal differences ($19.5 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 4.2$ S.E. in May versus $23.7 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 5.4$ S.E. in July). Sediments in the upper third of the lake showed higher rates during July, but not significantly so. Mean ambient denitrification (U-DEN) was low throughout the lake during May and several orders of magnitude higher during July (Figure 13). The elevated lake-wide mean during July resulted

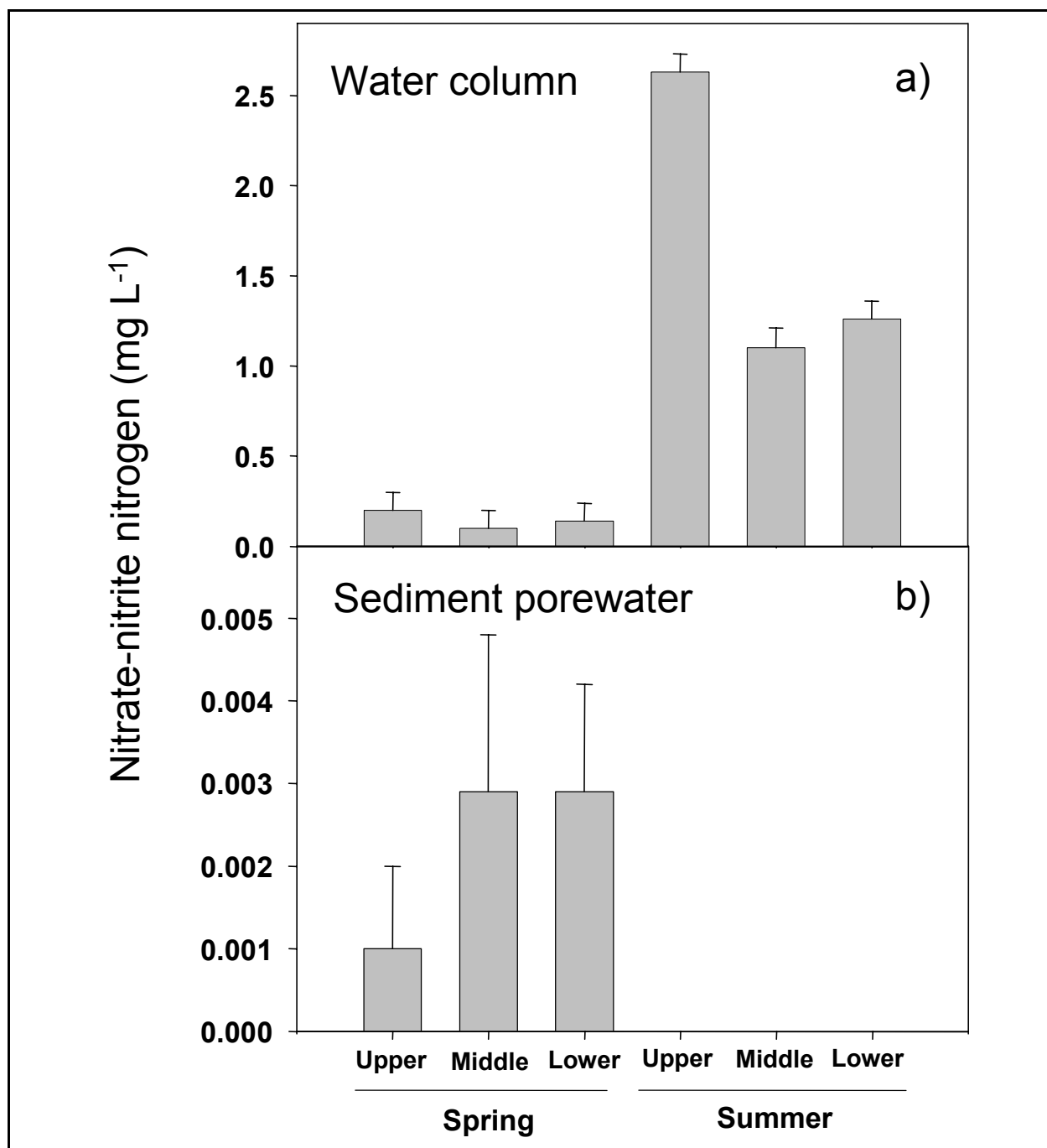


Figure 10. Mean water column (a) and porewater nitrate (b) in May (=spring) and July (=summer) 2004. Vertical line represents 1 standard error

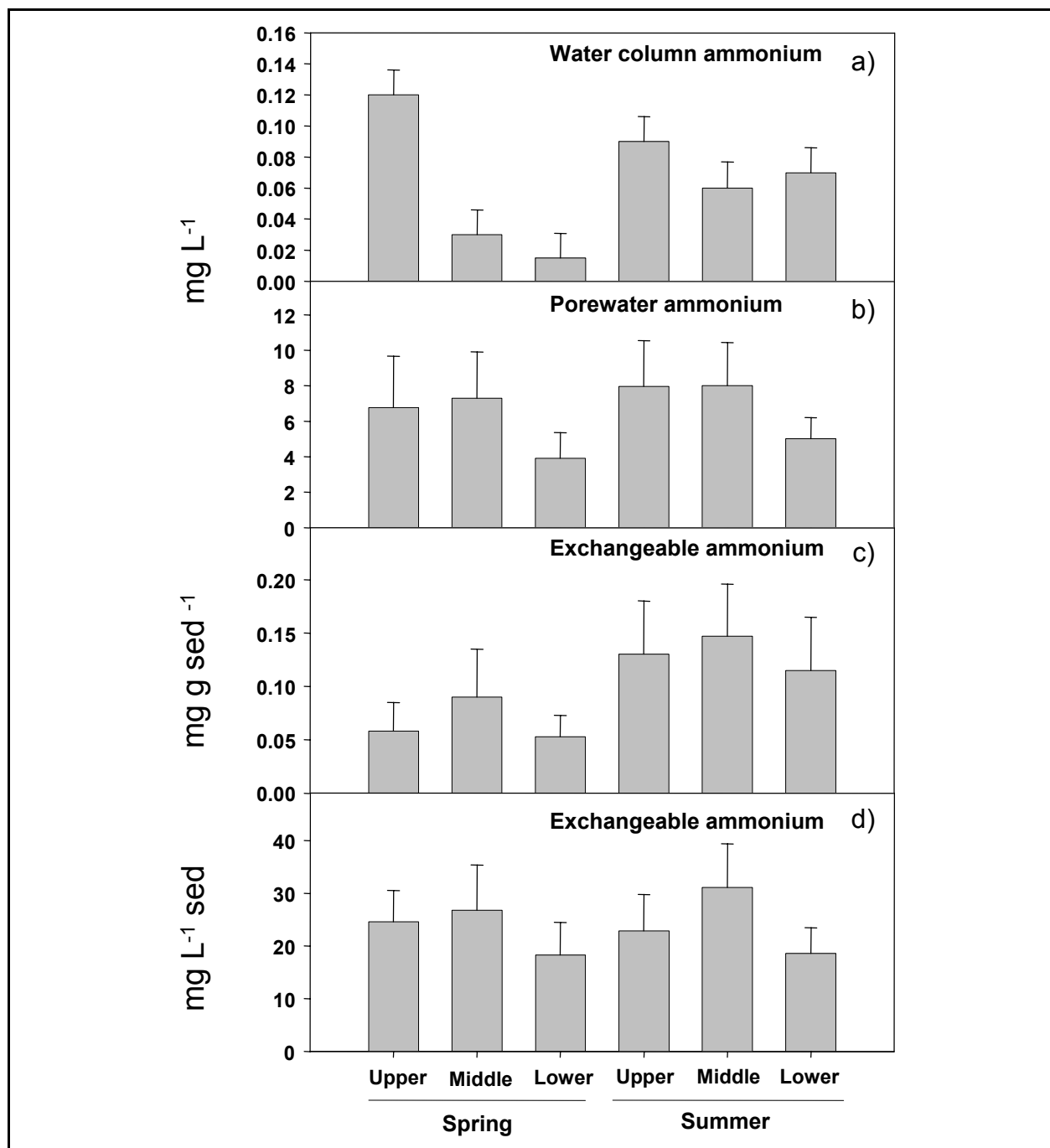


Figure 11. Mean water column and porewater concentrations of ammonium nitrogen in May (=spring) and July (=summer) 2004. Vertical line represents 1 standard error

Figure 12. Mean water column and porewater concentrations of ammonium nitrogen in May (=spring) and July (=summer) 2004. Vertical line represents 1 standard error

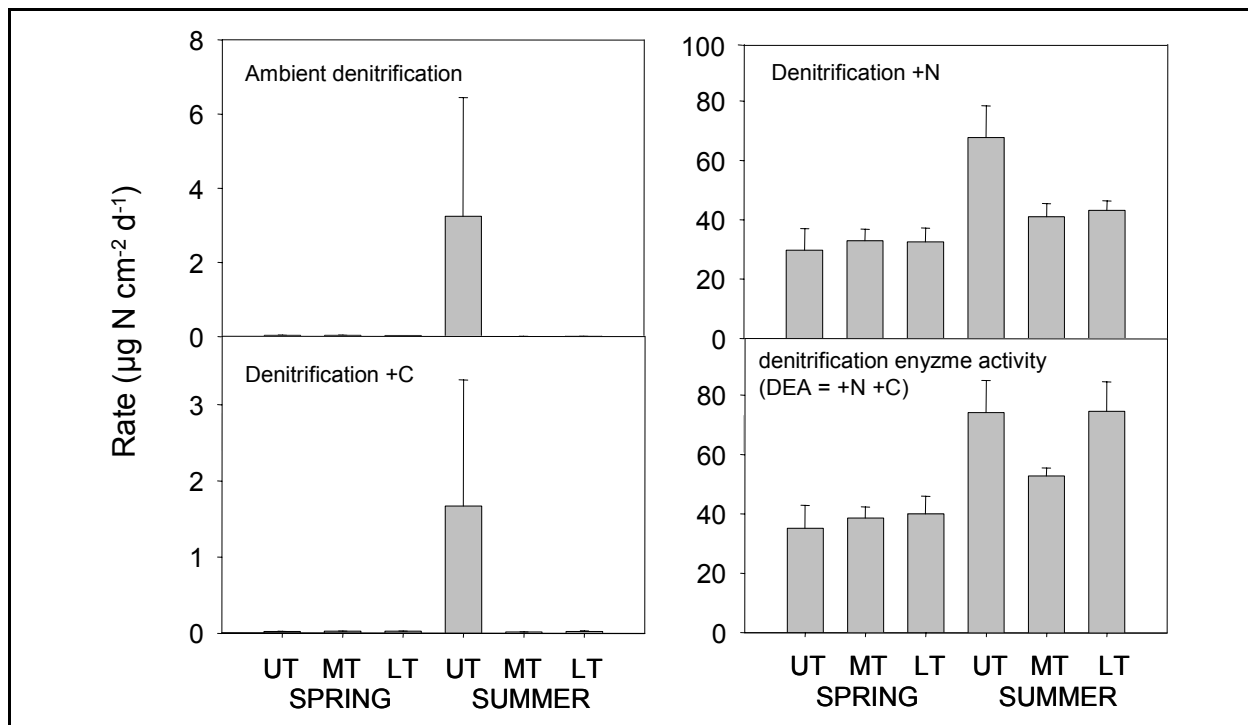
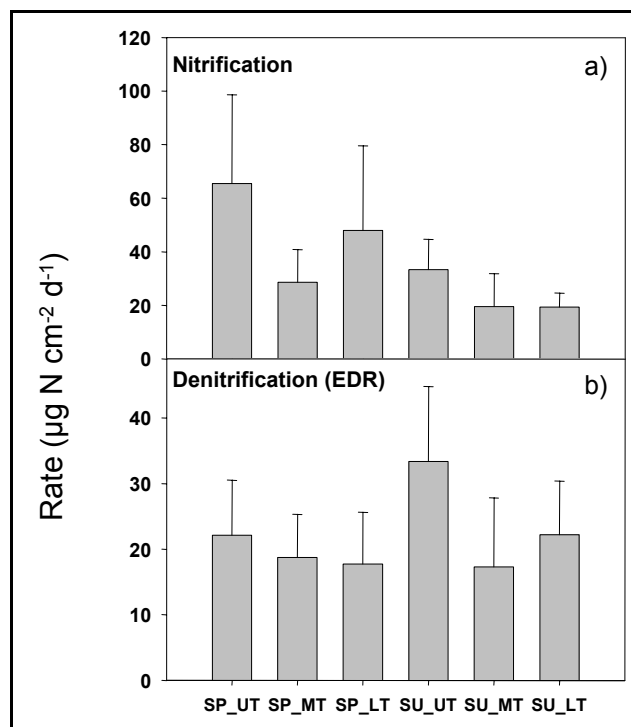


Figure 13. Mean rates of sediment denitrification with addition of excess carbon and nitrogen in Third Lake as a test of C and N limitation. Ambient = addition of site water only, +C = addition of glucose, +N = addition of nitrate, +C+N = addition of both glucose and nitrate. Spring = May, Summer = July 2004. UT= upper third of lake, MT=middle third, LT=lower third. See Methods section for details of methods. Vertical line represents 1 standard error

from extremely high rates in the upper third of the lake near the inflow culvert ($3.2 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 3.2 \text{ S.E.}$). Sediments in the lower two-thirds of the lake had extremely low rates in July ($\sim 0.01 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$). Carbon additions (Figure 13) had little effect on denitrification (Figure 13) except in the sediments from the upper portion of the lake during July, indicating little lake-wide C-limitation. Conversely, nitrate additions resulted in significant, nearly three orders of magnitude increase in denitrification ($31.7 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 3.0 \text{ S.E.}$) in May and significantly higher ($P < 0.0001$) rates in July ($50.7 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 4.8 \text{ S.E.}$). Nitrate-amended sediments from the upper third of the lake also exhibited much higher rates ($67 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 10.8 \text{ S.E.}$) than the rest of the lake ($43 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 3.9 \text{ S.E.}$). Additions of both C and N resulted in only a slight increase in rates over the N additions in the spring, but much higher rates in sediments from the lower third ($68.33 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} \pm 5.6 \text{ S.E.}$). These responses indicated strong nitrate limitation for denitrification in all locations during May and July, particularly in the upper third of the lake. Co-limitation with C and N was most striking in the lower third of the lake during July. Nitrification rates ranged from $43.7 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1} (\pm 14.2 \text{ S.E.})$ in May to $24.3 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$ in July ($\pm 5.6 \text{ S.E.}$; Figure 13). There were no significant consistent seasonal or spatial patterns.

Nitrate-Nitrite N Budget for Third Lake. When averaged across all dates and sites, EDR-derived nitrate removal was $\sim 32 \text{ kg} \cdot \text{d}^{-1}$ from the lake, which represented ~ 67 percent of the net $\text{NO}_3\text{NO}_2\text{-N}$ retention (Table 3). When internal nitrate additions via sediment nitrification ($\sim 51 \text{ kg} \cdot \text{d}^{-1}$) were factored into the budget, gross $\text{NO}_3\text{NO}_2\text{-N}$ retention was $99 \text{ kg} \cdot \text{d}^{-1}$, with denitrification accounting for ~ 32 percent. By subtraction, assimilation (bacteria, periphyton, phytoplankton, and macrophyte uptake) must have accounted for ~ 68 percent of the gross $\text{NO}_3\text{NO}_2\text{-N}$ retention ($\sim 67 \text{ kg} \cdot \text{d}^{-1}$).

Table 3		
Summer (May-September) Nitrate-Nitrite-N Budget for Third Lake¹		
Variables		Flux (kg d⁻¹)
Measured inputs	Culvert loading	113
	Nitrification	51
Measured outputs	Denitrification	32
	Lake discharge	65
Difference	Gross retention	99
	Net retention	48
¹ Denitrification rates = the EDR determination (Table 1). Net retention = culvert loading - lake discharge. Gross retention = (culvert loading + nitrification) - lake discharge.		

DISCUSSION: Third Lake was a sink for total N and $\text{NO}_3\text{NO}_2\text{-N}$ influxes from the Mississippi River during summer nominal flow periods in 2004. Summer net retention efficiencies of 31 and 43 percent for total N and $\text{NO}_3\text{NO}_2\text{-N}$ culvert loads, respectively, were comparable to N retention efficiencies measured for wetland complexes and freshwater diversion structures (Phipps and Crumpton 1994, Spieles and Mitsch 2000, Lane et al. 2004). When nitrification was factored into the overall N budget, $\text{NO}_3\text{NO}_2\text{-N}$ retention efficiency over the summer period was 60 percent. These results suggested that backwater regions of large river systems can be efficient in N retention and, if managed for optimal connection with N-rich main channel water, could play an important role in reducing N flux to coastal systems like the Gulf of Mexico. In contrast,

main channel N transformation and removal efficiency is typically much lower (5 to 20 percent, Seitzinger 1988, Richardson et al. 2004).

Biological uptake and permanent N loss from the system by denitrification accounted for a large percentage of the net $\text{NO}_3\text{NO}_2\text{-N}$ retention in Third Lake. Estimated denitrification rates (EDR) were much higher than ambient rates and accounted for the stimulatory effects of nitrate derived from nitrification. In situ production of nitrate via nitrification resulted in an additional internal load of 51 kg d^{-1} that was available for assimilation or denitrification. Of the total nitrate available in Third Lake, it is estimated that ~32 percent was denitrified and permanently lost from the system.

Experimental denitrification assays indicated that $\text{NO}_3\text{NO}_2\text{-N}$ delivery, not organic carbon availability, was limiting denitrification rates in Third Lake. This result was supported by the elevated denitrification rates coinciding with increased $\text{NO}_3\text{NO}_2\text{-N}$ concentrations measured during July (relative to May concentrations). It was also supported by the elevated denitrification rates in sediments from the region directly downstream from the inflow culvert, which receives the highest concentration of $\text{NO}_3\text{NO}_2\text{-N}$. It is likely that nitrate depletion downstream from the inflow culvert limited denitrification throughout the lower two-thirds of the lake. This is suggested by the longitudinal pattern of exponential decay in $\text{NO}_3\text{NO}_2\text{-N}$ concentration. Under these input conditions, net $\text{NO}_3\text{NO}_2\text{-N}$ retention efficiency declined in a curvilinear pattern as N loading increased, suggesting that biological uptake was becoming saturated and/or physical mechanisms, such as nutrient contact time and delivery via diffusive N flux into the sediment, were becoming limiting factors in denitrification. In particular, the N uptake length approached the length of the lake as loading increased above 200 kg d^{-1} , suggesting that rate-limiting diffusion of $\text{NO}_3\text{NO}_2\text{-N}$ into the sediment probably limited denitrification in the downstream regions of the lake. Nevertheless, overall N mass retention increased as a function of N culvert loading throughout the entire range of N culvert loading rates discharged into Third Lake during the summer of 2004. N mass retention and efficiency would be expected to level off and perhaps begin to decline at very high N culvert loadings (i.e., higher than observed in 2004), due to complete saturation of the system with N. Yet, even at this asymptotic level, N retention could average around 40 percent (Figure 6), a substantial amount given the tremendous loads fluxing through the Upper Mississippi River.

The assimilated nitrogen ($\sim 67 \text{ kg} \cdot \text{d}^{-1}$) was likely incorporated into temporary storage for later release and processing or transport. Phytoplankton and periphyton can assimilate considerable nitrate for growth in the absence of ammonia in freshwater systems (Chan and Campbell 1978). In particular, the presence of submersed and floating-leaved aquatic macrophytes in Third Lake probably played an important indirect role in N retention by providing substrate for periphyton growth. Transformation of $\text{NO}_3\text{NO}_2\text{-N}$ into periphyton biomass in backwater systems would also lead to some permanent N loss from the system via bacterially mediated detrital recycling pathways (algal decomposition > nitrification > denitrification). However, a portion of this N pool could also leave the system as recycled soluble species during winter and spring flooding and represent a load to downstream reaches, thereby reducing overall N retention and dissipation efficiency. An additional 21.5 kg N ($\sim 0.2 \text{ kg} \cdot \text{d}^{-1}$) was incorporated into submersed macrophyte tissue during the summer, but it is likely that this N pool was derived from the sediment (porewater and sediment exchangeable $\text{NH}_4\text{-N}$) by root uptake rather than from the water column

(Barko et al. 1991, Barko and James 1998). It is, thus, unlikely that submersed macrophytes played a direct role in net N removal capacity or efficiency of Third Lake over the relatively short period of this study. As with phytoplankton and periphyton biomass, the macrophyte N pool is subject to N transformation, recycling, dissipation, and downstream transport as a result of decomposition in the autumn and early winter.

A small net export of $\text{NH}_4\text{-N}$ from Third Lake occurred throughout most of the summer. However, this loss was minor compared to other N fluxes. This export may be attributed to net flux of $\text{NH}_4\text{-N}$ out of the sediment in excess of its conversion to nitrate via nitrification. Porewater and exchangeable sediment concentrations of $\text{NH}_4\text{-N}$ measured in this study were high (overall mean = 6.46 and $18.65 \text{ mg} \cdot \text{L}^{-1}$, respectively) and other work has shown that laboratory-derived rates of $\text{NH}_4\text{-N}$ release from the sediment are high at $19.6 \text{ mg m}^{-2} \cdot \text{d}^{-1}$ ($\pm 5.5 \text{ S.E.}$) under oxic conditions and $53.4 \text{ mg m}^{-2} \cdot \text{d}^{-1}$ ($\pm 3.4 \text{ S.E.}$) under anoxic conditions (James, unpublished data). The higher, anoxic rates of $\text{NH}_4\text{-N}$ release are probably frequent in backwaters like Third Lake. Diel fluctuations in dissolved oxygen, mediated by dense macrophyte stands, inhibit nitrification at the sediment interface during the night and result in net flux of $\text{NH}_4\text{-N}$ out of the system. During the day, intense photosynthesis and oxic conditions will encourage the nitrification of sediment-derived $\text{NH}_4\text{-N}$ and provide the nitrate needed for denitrification when nitrate delivery from external sources is restricted.

N retention and dissipation in large river systems may be improved by increased connections between backwaters and the main channel. For backwaters that can receive inputs by controlled flows, N retention efficiency and capacity to process N loads can be optimized by considering flow, load (over a range of N concentrations), and water residence time in relation to biological uptake, nitrification, denitrification, and recycling in the design of the controlled flow structures. Large river and receiving-water models of nitrogen dynamics are needed to design the flow of water into backwaters. These same models can help forecast the impact of routing water through backwaters on overall in-stream N processing and fate of N loads being transported downstream. Denitrification in the available backwaters of the Upper Mississippi River may not be able to substantially reduce the high $\text{NO}_3\text{NO}_2\text{-N}$ loads now received by the main stem from the surrounding watershed (Richardson et al. 2004). However, other mechanisms of nitrogen retention are still unquantified. In particular, the role that submersed aquatic vegetation may play in N dynamics, by acting as substrate for periphyton growth, by altering dissolved oxygen and redox conditions for nitrifiers and denitrifiers both in the water column (Caffrey and Kemp 1992) and at the root-sediment interface (Moorhead and Reddy 1988, Bodelier et al. 1996), and by providing organic carbon and N to detrital pathways for increased N processing and dissipation, is still unknown. These and other macrophyte-mediated processes need to be incorporated into large river and receiving-water models to improve forecasting capabilities and system-level management.

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